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1 **Ureteral morphology and pathology during urolithiasis in cats**

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18  
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24

25 **Abstract**

26 Cats show high susceptibility to the urinary organ-related diseases. This study investigated the  
27 morphological characteristics of healthy ureters and compared them with surgically resected ureters  
28 distal to the lesion obstructed by urolithiasis in cats. Healthy ureters (total length  $9.88 \pm 0.38$  cm)  
29 developed adventitia composed of collagen fibers (ADCF), containing longitudinal muscular layer,  
30 toward the distal segment. Healthy ureter was smallest in the middle segment (4.71–6.90 cm from  
31 the urinary bladder) with significantly decreased area of its lumen and submucosa from the proximal  
32 segment. Diseased cats showed a high incidence of calcium oxalate (CaOx) urolithiasis with renal  
33 dysfunction, regardless of age, sex, and body size. Their ureters showed increased perimeters,  
34 inflammation, and decreased nerves in ADCF. Collagen fibers were increased in the submucosal area,  
35 intermuscular spaces, and ADCF, especially near the obstructed lesion. The mean resected ureter  
36 length was  $5.66 \pm 0.49$  cm, suggesting a high obstruction risk in the middle segment. The middle  
37 segment also increased the cross area of ureter and ADCF, regardless of the distance from the  
38 obstructed lesion. Importantly, the ureters of several cases showed a lack of transitional epithelium  
39 or its hyperplasia, and some of them formed the mucosal folds. Thus, we report the characteristics  
40 and histopathological features of cat ureters; especially, the decreases of ureter size, lumen area,  
41 and submucosa area from proximal to middle segment in healthy and ADCF alternations in  
42 urolithiasis, including increased connective tissues with inflammation and decreased nerves, would  
43 be crucial to consider the pathogenesis of feline ureteral obstruction.

44

45 **Running head:** Cat ureter and urolithiasis

46

47 **Keywords:** Cat, ureter, histology, histopathology, calcium oxalate, urolithiasis

48

49 **Highlights**

- 50 ● HC ureter was smallest in the middle segment (4.7–6.9 cm from the urinary bladder)
- 51 ● Ureter with feline urolithiasis (UWFU) showed increased perimeters and inflammation
- 52 ● UWFU decreased nerves in adventitia composed of collagen fibers
- 53 ● Histological data suggested the high obstruction risk in the middle segment of UWFU

54 **Introduction**

55 In small animal veterinary medicine, large survey data reported that approximately 4% of dogs and  
56 12% of cats die from a renal disorder in UK (Lewis et al., 2018; O'Neill et al., 2015). In particular, data  
57 from Europe and the USA indicated that 4.6% of cats in private practices, and 7% to 8% of those in  
58 veterinary teaching hospitals show feline lower urinary tract disease (FLUTD), manifesting as  
59 pollakiuria, hematuria, stranguria, or complete or incomplete urinary tract obstruction (Dru  
60 Forrester and Roudebush, 2007). FLUTD cases with urinary tract obstruction can be cause uremia  
61 and hyperkalemia, the mortality of which reaches 5% (Kaul et al., 2020). FLUTD is caused by bacterial  
62 infections, urethral plugs, anatomical defects, neoplasia, idiopathic cystitis, and urolithiasis (Dru  
63 Forrester and Roudebush, 2007; Kaul et al., 2020; Lulich et al., 2016).

64 In a German cat population, urolithiasis is observed in 7.0% of animals with FLUTDs ( $n = 302$ )  
65 (Dorsch et al., 2014). The main components of urinary calculus are calcium oxalate (CaOx) or struvite  
66 in dogs and cats. In cats, a Canadian study reported that 49% or 43% of bladder uroliths were  
67 composed of struvite or calcium oxalate, respectively (Houston and Moore, 2009). In particular,  
68 feline urolithiasis caused by CaOx is increasing due to the aging of individuals and promotion of urine  
69 acidification or animal protein-containing diets (Lulich et al., 2016; Osborne et al., 2009).  
70 Hypercalcemia caused by primary hyperparathyroidism or feline idiopathic hypercalcemia is another  
71 known risk factor for CaOx urolithiasis (Finch, 2016; Lulich et al., 2016). CaOx is insoluble, and it is  
72 important to prevent its formation; to this end, potassium citrate and thiazide diuretics have been  
73 used for therapeutic purposes (Lulich et al., 2016; Osborne et al., 2009). CaOx obstructing the lower  
74 urinary tract, including the urinary bladder or urethra, can be eliminated by minimally invasive  
75 procedures such as lithotripsy or basket retrieval (Lulich et al., 2016). However, the upper urinary  
76 tract, including the renal pelvis or ureters, should be immediately eliminated by surgical procedures,  
77 such as stents, bypasses, ureterotomy, and resection and anastomosis, because it can cause serious  
78 symptoms or irreversible morpho-functional alternation of kidneys and ureters, ultimately leading  
79 to hydronephrosis or hydroureter (Berent et al., 2018; Lulich et al., 2016).

80 The elucidation of species-specific morphological features in each organ would improve the  
81 understanding of the relationship between anatomy, physiology, and pathology. Given that cats  
82 show high susceptibility to urinary tract diseases, including urolithiasis, the clarification of their

83 pathology is crucial in the field of veterinary medicine. Almost all previous studies have mainly  
84 focused on the pathological effects of diet or animal health status, such as infection, endocrinopathy,  
85 and congenital urinary tract disorders, as risk factors for cat urolithiasis (Brouman, 2011; Dru  
86 Forrester and Roudebush, 2007; Houston and Moore, 2009; Kaul et al., 2020; Lulich et al., 2016;  
87 Nesser et al., 2018; Osborne et al., 2009, 1979). However, the normal histological structures and  
88 pathological alternations of cat ureters have not been fully investigated.

89 In this study, we examined the normal histological features of cat ureters in several  
90 compartments. Moreover, the histopathological changes due to urolithiasis were examined in  
91 surgically resected ureters. Our results provide a crucial basis for understanding the anatomical and  
92 physiological characteristics, as well as the pathogenesis of cat ureters.

93

94 **Materials and Methods**

95 ***Sample preparations from healthy cats***

96 The age and body weight (BW) of the animals were recorded before sampling. We obtained the  
97 ureters from healthy cats ( $n = 4$  animals, 8 ureters,  $1.08 \pm 0.04$  years), which were euthanized for use  
98 in other experiments approved by the Institutional Animal Care and Use Committee of the Faculty  
99 of Veterinary Medicine, Hokkaido University (approval no. 14-0054, 20-0081). The obtained ureters  
100 were fixed with 10% neutral buffered formalin (NBF).

101

102 ***Definition of ureter segments in healthy cats***

103 Images of fixed ureters were obtained, and their lengths were measured using ImageJ software  
104 (National Institutes of Health, Bethesda, MD, USA). The obtained ureters were divided into four  
105 segments and defined as proximal (Pro), middle 1 (Mid1), middle 2 (Mid2), and distal (Dis); the actual  
106 distance or the range of segments were more than 6.91 cm, 4.71–6.90 cm, 2.31–4.70 cm, and 0.00–  
107 2.30 cm from the ureter-urinary bladder junction, respectively.

108

109 ***Sample preparations from cat clinical cases***

110 Informed consent was obtained from the owners of the animals. The age and BW of the cats were  
111 recorded, and blood urea nitrogen (BUN) and serum creatinine (CRE) were measured using a Fuji  
112 Dri-Chem biochemistry analyzer (Fujifilm, Tokyo, Japan). The ureters ( $n = 20$  cats, 24 ureters) were  
113 obtained from cat cases manifesting obstructive urolithiasis in Matsubara Animal Hospital  
114 (Matsubara, Japan) that resulted in surgical therapeutic resection. The nature of the urinary calculus  
115 was analyzed by the infrared absorption spectroscopy in Osaka Kessei Research Laboratories, Inc.  
116 (Osaka, Japan). The resected ureters contained from just proximal portion of obstructed lesions to  
117 the ureter-urinary bladder junction, and they were fixed using 10% NBF.

118

119 ***Definition of ureter segments in cat clinical cases***

120 Diseased ureters were divided into four equal parts termed disease parts (dPart) 1–4. The length of  
121 each dPart depended on the localization of obstructive lesion and differed among cat cases. Each  
122 dPart was mapped to the corresponding segment of the healthy ureter. Briefly, to judge the

123 correspondence between each dPart and the criteria in healthy ureter segments (Pro, Mid1, Mid2,  
124 Dis), the distance from ureter-urinary bladder junction of each dPart was measured and  
125 corresponded to the actual distance or the range of segments (6.91 cm, 4.71–6.90 cm, 2.31–4.70  
126 cm, and 0.00–2.30 cm) defined in healthy cats. Further, diseased ureters containing obstructed  
127 regions (dUOR) or its distal regions (dUDR) were separately evaluated in histopathological analysis.

128

### 129 ***Histological analysis***

130 Fixed ureters were dehydrated using alcohol and were embedded in paraffin. Deparaffinized  
131 histological cross-sections (2–3- $\mu$ m-thick) were stained with Masson's trichrome (MT) or  
132 immunohistochemistry (IHC). For IHC, sections were incubated in 20 mM Tris-HCl buffer (pH 9.0) for  
133 15 min at 110°C, treated with 0.3% H<sub>2</sub>O<sub>2</sub>/methanol solution for 20 min, and blocked using 10%  
134 normal goat serum (SABPO kit; Nichirei Bioscience, Tokyo, Japan). Sections were incubated overnight  
135 with primary antibodies for CD20 (B cell marker; 1:300; E2560, Spring Bioscience, Pleasanton, CA,  
136 USA) or IBA1 (macrophage marker; 1:800; 019-19741, FUJIFILM Wako Pure Chemical Corporation,  
137 Osaka, Japan) at 4°C. The sections were then treated with biotinylated goat anti-rabbit IgG (SABPO  
138 kit; Nichirei Bioscience) for 30 min at room temperature. This was followed by incubation with  
139 streptavidin-horseradish peroxidase using the SABPO kit (Nichirei Bioscience) for 30 min, followed  
140 by incubation with 3,3-diaminobenzidine tetrahydrochloride. Finally, the sections were  
141 counterstained with hematoxylin.

142

### 143 ***Histoplanimetry***

144 All stained sections were examined using a BZ-X710 microscope (Keyence, Osaka, Japan) and  
145 converted to virtual slides using a NanoZoomer 2.0-RS (Hamamatsu Photonics, Shizuoka, Japan). By  
146 using MT-stained sections, each component of the ureters, including the adventitia, muscular layer  
147 (ML), submucosa (SM), transitional epithelium (TE), and lumen (LU), was analyzed. As the adventitia  
148 was compartmentalized into external large and internal narrow layers composed of adipose tissue  
149 and collagen fibers, respectively, we focused on the latter, "adventitia composed of collagen fibers  
150 (ADCF)." The perimeters of ADCF or ML were measured as thickness indices of the ureters by  
151 NDP.view2 (Hamamatsu Photonics). The area of each ureter component was also measured by

152 NDP.view2, and the percentage of the total ureter area was calculated. For ML development, internal  
153 longitudinal ML (Int LL), middle circular ML (Mid CL), external longitudinal ML (Ext LL), and  
154 longitudinal ML found in the ADCF (Ad LL) were identified in the sections, and their development  
155 was categorized as follows: Glade 0, undeveloped; Glade 1, identifiable but less-developed; Glade 2,  
156 clearly identifiable and developed; and Grade 3, clearly identifiable and well-developed. The average  
157 grade in the examined sections was expressed as a score for ML development. Further, the number  
158 of nerve bundles found in MT-stained sections, and CD20<sup>+</sup> or IBA1<sup>+</sup> cells in IHC sections were counted  
159 in the ureter, and their numbers/examined ureter areas were calculated.

160

#### 161 ***Statistical analysis***

162 All statistical analysis was performed by non-parametric methods using SPSS statistics version 23  
163 (IBM Japan, Ltd., Tokyo, Japan). Mann–Whitney *U*-test was used for two-group comparisons. For  
164 multiple comparisons, Dunnett's test or Scheffé's method was performed when statistical  
165 significance was observed with the Kruskal–Wallis test. Statistical significance was set at  $P < 0.05$ .

166

167 **Results**

168 ***Body weight and ureter morphology of healthy cats***

169 Table 1 summarizes the information of the examined healthy cats. Body weight, left ureter length,  
170 and right ureter length were  $4.32 \pm 0.18$  kg,  $8.50 \pm 0.35$  cm, and  $9.88 \pm 0.38$  cm, respectively. The  
171 mean of left and right ureter length was  $9.19 \pm 0.34$  cm.

172 Histologically, the ureter mucosa was composed of TE and SM, and the lamina muscularis was  
173 not present in all segments (Fig. 1A). Well-developed Mid CL and less-developed Int LLs and Ext LLs  
174 were observed. ADCF attached to the most external ML, and their development differed among  
175 examined ureter segments (Fig. 1A). The outside of the ADCF was covered by adipose tissue in the  
176 adventitia. For indices of ureter thickness, the ADCF and CL perimeters were 2.7 and 2.3 mm (Fig.  
177 1B, "Total" showing the mean of all examined segments), and they were similar values among the  
178 examined segments. The measured area of each ureter component differed among segments,  
179 especially SM in Mid1 or Mid2 was smaller but ADCF in Dis was larger than their corresponding  
180 components in Pro (Fig. 1C). Importantly, area of LU was significantly smaller in Mid1 than in Pro  
181 when they were analyzed by two-group comparisons (Mann–Whitney *U*-test,  $P = 0.02$ ) although  
182 there was no significant difference in multiple comparisons. Figure 1D shows the percentage  
183 component of the total ureter area, and SM in Pro had a significantly higher ratio than the other  
184 segments. Furthermore, ADCF in Mid2 or Dis had a significantly higher ratio compared to Pro, but  
185 there was no segment-related difference in Lu, TE, and CL.

186 Figure 2 focuses on ML development. The Int LLs and Ext LLs were quite thin and partially  
187 observed in all examined ureter regions, whereas longitudinal muscular fibers were also observed  
188 in ADCF, termed Ad LL (Fig. 2A). With regard to the morphometry of ML in the entire ureter, the Mid  
189 CL was significantly better developed compared to the other layers in both species, and the cat Ad  
190 LL was also significantly better developed compared to the Int and Ext LLs (Fig. 2B). For comparison  
191 of ML development, the Mid CL in Dis and the Ad LLs in Mid1, Mid2, and Dis were significantly better  
192 developed compared to the corresponding MLs in Pro (Fig. 2C). Moreover, the Ad LL in Dis also  
193 showed a significantly higher score than Mid1. These data emphasize the morphological differences  
194 among cat ureter segments.

195

196 ***Clinical and ureter information of diseased cats***

197 Table 2 summarizes the information on cats with ureteral urolithiasis. Of the eight males and 12  
198 females examined, only one female was intact, and the others were neutered. Almost all cases  
199 manifested CaOx urolithiasis, and dried solidified blood stone, magnesium ammonium phosphate,  
200 or ammonium acid urate were also detected in one case each. There was no sex-related differences  
201 in age (male vs female;  $6.56 \pm 1.31$  vs  $7.97 \pm 0.93$  years,  $P = 0.427$ ), BW ( $3.84 \pm 0.22$  vs  $3.63 \pm 0.29$   
202 kg,  $P = 0.343$ ), BUN ( $77.96 \pm 17.42$  vs  $97.22 \pm 21.39$  mg/dL,  $P = 0.851$ ), CRE ( $4.36 \pm 1.16$  vs  $7.42 \pm 2.01$   
203 mg/dL,  $P = 0.571$ ), or dissected ureter length ( $5.55 \pm 0.60$  vs  $5.74 \pm 0.74$  cm,  $P = 1.000$ ).

204

205 ***Histopathological features and size of diseased cat ureters***

206 Diseased ureters were divided into four equal parts (dPart, Fig. 3A), and their histopathological  
207 differences were examined. Diseased ureters showed a dilated lumen (Fig. 3B) and increased  
208 connective tissues from the SM to the ADCF (Fig. 3C), and these features differed among individual  
209 cats or examined parts. For histopathological comparison of each part, dPart1 was the thickest; it  
210 decreased toward dPart4, and significance was detected between dPart1 and dPart4 in the ADCF  
211 perimeter (Fig. 3D). Further, the area of each component showed a similar tendency with perimeter,  
212 and the SM and CL in dPart1 were significantly larger than those in dPart4; there was no part-related  
213 differences in LU, TE, and ADCF (Fig. 3E). There were no significant differences in the percentage of  
214 total ureter area among the examined parts (Fig. 3F). These data indicate that dPart1, a part close  
215 to the obstructed lesion due to urolith, showed a tendency for thickening without the alternation of  
216 each composition ratio.

217

218 ***Comparison of ureter size between healthy and diseased cats***

219 Each part of the diseased ureter was mapped to the corresponding segment of the healthy ureter,  
220 and dUOR or its distal regions, dUDR, were separately evaluated (Fig. 4A). The mean of all examined  
221 segments was calculated, and the diseased ureters were found to be significantly thicker than  
222 healthy ureters (Fig. 4B). As for the perimeter of each segment, all examined segments in dUORs  
223 tended to show higher values compared to healthy ureters, and significance was detected in the  
224 ADCF and CL of Mid1, Mid2, and Dis. The dUDRs showed significantly higher values in the ADCF of

225 Mid1 and Mid2, and the CL of Mid1. These data indicate the presence of thickened diseased ureters,  
226 particularly near obstructed lesions.

227

#### 228 ***Comparison of each component area between healthy and diseased cat ureters***

229 With the exception of LU, the area of all components was significantly larger in diseased ureters than  
230 in healthy ureters (Fig. 5A). In terms of the percentage of each ureter component, the ADCF was  
231 larger, but the other components were significantly smaller in diseased ureters than in healthy  
232 ureters (Fig. 5B). For area comparison in each segment (Fig. 5C), dUORs showed significantly larger  
233 ADCF in Pro; ADCF, CL, or SM in Mid1; ADCF or SM in Mid2; and CL or SM in Dis compared to healthy  
234 ureters. The dUDRs showed a significantly larger CL in Mid1 than in the healthy ureter. For  
235 percentage expression (Fig. 5D), the dUOR showed significantly larger ADCF and smaller SM or LU in  
236 Pro; and larger ADCF and smaller SM or TE in Mid1 compared to healthy ureters. Moreover, the  
237 dUDRs showed significantly larger ADCF and smaller SM in Mid1, larger ADCF in Mid2, and larger  
238 ADCF and smaller SM in Dis compared to healthy ureters.

239

#### 240 ***Comparison of each ML between healthy and diseased cat ureters***

241 The mean of all examined segments was calculated, and diseased ureters showed significantly higher  
242 scores for ML development in Int, Ext, and Ad LLs than in healthy ones (Fig. 6A). For each region,  
243 dUOR showed significantly better developed Ad LL in Pro; Int or Ext LL in Mid1; and Ext LL in Mid2  
244 compared to healthy ureters (Fig. 6B). Furthermore, dUDR showed better developed Int or Ext LL in  
245 Mid2 compared to healthy ureters. Furthermore, dUDR showed better developed Int LL compared  
246 to dUOR.

247

#### 248 ***Inflammatory features of diseased cat ureters***

249 In diseased ureters, CD20<sup>+</sup> B cells and IBA1<sup>+</sup> macrophages infiltrated the ADCF, and the latter were  
250 also observed in the SM and TE (Fig. 7A). There was no significant difference in the number of  
251 inflammatory cells between each part of the diseased ureter, although dPart1 and dPart2 tended to  
252 show high values (Fig. 7B). From the mean of all examined segments, the diseased ureter showed  
253 significantly higher CD20<sup>+</sup> B cell numbers than the healthy ureter, although IBA1<sup>+</sup> macrophages were

254 abundant in both ureters (Fig. 7C). For each segment, the number of CD20<sup>+</sup> B cells tended to be  
255 higher in dUORs and dUDRs than in healthy ureters, and significance was detected in Pro and Dis of  
256 dUOR (Fig. 7D). No significant increase in urolithiasis was observed in IBA1<sup>+</sup> macrophages in Pro,  
257 Mid1, and Mid2, but that in Dis of dUORs was significantly higher than that in healthy ureters (Fig.  
258 7E).

259

#### 260 ***Decreased nerve bundles and unique histopathological features of diseased cat ureters***

261 Nerve distributions are crucial for the sensation and movement of the ML. In healthy ureters, nerve  
262 bundles were observed in the ADCF, but these were decreased in the ADCF of diseased ureters (Fig.  
263 8A). Moreover, the number of nerve bundles tended to increase from dPart1 to dPart4 (from the  
264 obstructed region to its distal regions) in diseased ureters, whereas it was significantly decreased in  
265 diseased ureters compared to healthy ureters in mean from all examined segments (Fig. 8B). A  
266 similar significant decrease in the diseased ureter was detected in each region, regardless of the  
267 obstructed regions (Fig. 8C).

268 In terms of other pathological characteristics of diseased ureters, the decudation of TE, its  
269 invasion and invagination toward ML, and the formation of mucosal folds were also observed (Fig.  
270 8D).

271

272 **Discussion**

273 In healthy cat ureters, the Mid CL is the most developed ML among the entire ureter, and its  
274 contraction and relaxation mainly contributes to ureteral peristalsis and urine transport (Kiil, 1973).  
275 However, in three-dimensional features, ureter MLs run by oblique patterns; therefore, the  
276 combined movement of the Mid CL, as well as longitudinal muscular bundles on histological sections  
277 might be crucial for urine transportation by ML. Healthy cats show development of ADCF and Ad LL  
278 toward ureter-urinary bladder junction, indicating segmental differences in ureter movement. These  
279 anatomical and histological features might affect the transport efficiency of urine from the renal  
280 pelvis to the urinary bladder. Food and obesity are considered risk factors for urinary organ-  
281 associated diseases, including urolithiasis in cats (Gomes et al., 2018; Kocabağlı et al., 2017). In  
282 addition, morphological characteristics of ureters, such as differences in relative length and width,  
283 or the development of ML and ADCF might affect their pathogenesis.

284 Diseased cats showed a high incidence rate of urolithiasis due to CaOx calculus. Recently, the  
285 incidence of urolithiasis in cats has been increasing due to the aging of animals and the promotion  
286 of urine acidifying or animal protein-containing diets (Lulich et al., 2016; Osborne et al., 2009).  
287 Furthermore, the present study targeted urolithiasis cases that require surgical dissection of ureters;  
288 therefore, cases with CaOx were inevitably included. We found no relationship between urolithiasis  
289 development and age, sex, or BW, although these factors are considered to be factors affecting its  
290 incidence (Gomes et al., 2018; Kocabağlı et al., 2017). Furthermore, there was no significant  
291 correlation between elevated BUN or Cre and age, BW, sex, and dissected ureter length in diseased  
292 cats. Therefore, ureteral obstruction of CaOX calculus causes renal dysfunction regardless of age, BW,  
293 sex, and the localization of the obstructive region along the ureters.

294 The diseased cat ureters showed dilated or fibrotic features, which differed among the cases.  
295 In common findings, diseased ureters significantly increased perimeters due to the enlarged area of  
296 each component, developed LLs, abundant inflammatory cells, and decreased nerve bundles in ADCF.  
297 In particular, an increase in the ADCF perimeter and area of the SM and CL were observed in dPart1,  
298 a part close to the obstructed lesion. MT staining also revealed increased collagen fibers in SM,  
299 intermuscular spaces, and ADCF in diseased ureters, and these connective tissue developments also  
300 affect their area increase in dPart1. During the progression of fibrosis, inflammation also affects its

301 pathogenesis in various organs (Distler et al., 2019); however, we found no significant difference in  
302 inflammation scores among dParts1–4, although a decreasing tendency from dPart1 to dPart4 was  
303 observed. Therefore, in addition to inflammation, other factors, such as physical stimulation or  
304 pressure by CaOx calculus, cause severe alterations in ureter morphology in dPart1.

305 The mean value of the dissected ureter length is  $5.66 \pm 0.49$  cm, and our results suggest that  
306 Mid1 (4.71–6.90 cm) has the highest risk of CaOx calculus obstruction. In the healthy cat ureter, the  
307 perimeter and total area were smallest in Mid1 among the examined segments; in particular, the SM  
308 area was significantly smaller in Mid1 than in Pro. The ureter SM has developed collagen fibers and  
309 contributes to the adaptation to mucosal morphological changes associated with ureteral movement  
310 (Kiil, 1973; Takaddus et al., 2016). Furthermore, LU area was significantly decreased from Pro to Mid1  
311 in two group comparisons in healthy cat ureters. Therefore, the size decrease of the ureter, LU, and  
312 SM from Pro to Mid1 might create an environment that is prone to blockages of CaOx calculus.

313 As for the difference in histopathological features in each ureter segment, Mid1 showed  
314 remarkable morphological changes, such as ureter size indices and each component area in both  
315 dUOR and dUDR. These data suggest that Mid is sensitive to alterations in its structures in both Pro  
316 and Mid1 obstructions. Further, the area from ADCF to SM tended to increase, regardless of the  
317 segments or obstruction localizations. Characteristically, Ad LL was clearly observed in healthy cats  
318 from Mid1 onward, and longitudinal muscular layers tended to develop more frequently in diseased  
319 cats compared to healthy cats. Therefore, rather than the development of individual longitudinal  
320 muscle itself, the longitudinal muscle layer appears to develop as the developed connective tissue  
321 separates the oblique muscle bundles during urolithiasis. In particular, the ADCF increase seemed to  
322 principally contribute to this process. Furthermore, nerve bundles observed in ADCF were  
323 significantly decreased in diseased cats, regardless of the localization of the segment or obstruction.  
324 Ureters innervate the sympathetic nerves from the thoracic or lumbar spinal cords, parasympathetic  
325 nerves from the vagus nerve or pelvic nerve, and sensory nerves (Elbadawi and Schenk, 1969; Feher  
326 et al., 1981). Therefore, the nerve decrease is associated with the ADCF alternations with urolithiasis,  
327 and may also be associated with nerve dysfunction.

328 We also revealed TE-related alterations in urolithiasis. In general, CaOx stones show rough  
329 surface (Khan et al., 2016); therefore, it might physically damage the ureteral TE. In fact, the human

330 patients with upper urinary tract urolithiasis had significantly lower expression of tight junction  
331 proteins including E-cadherin and tight junction protein 1 with morphological alternations (Jiang and  
332 Kuo, 2014). Importantly, lack of TE was observed in several diseased cat ureters, indicating urine  
333 leakage to the ureter parenchyma. Urine-leakage has not been evaluated in urolithiasis ureters but  
334 is usually observed in trauma of the kidney, ureters, urinary bladder, and urethra (Moore et al.,  
335 2002; Titton et al., 2003), and in the obstructed renal pelvis (Mitchinson and Bird, 1971).  
336 Furthermore, TE-invasion to the adventitia, and the formation of longitudinal mucosal folds were  
337 observed in cat cases. The proliferative features of TE are observed in polypoid/papillary cystitis  
338 caused by a reactive proliferative lesion, and they are associated with irritation or injury from calculi,  
339 urinary outflow obstruction, ischemia, and inflammatory conditions (Samaratunga et al., 2021).  
340 Papillary urothelial hyperplasia/urothelial proliferation is also known to occur in the urinary bladder  
341 (Samaratunga et al., 2021). Although there are few reports about similar pathological changes in  
342 ureters, the histopathological features found in cat urolithiasis might be related to these TE  
343 alterations. In cases showing TE invasion to the adventitia, surgical procedures involving elimination  
344 of adipose tissues during resection of the ureter should consider the preservation of ADCF to avoid  
345 ureter perforation.

346 Taken together, our study clarified the morphological characteristics of cat ureters, and their  
347 histopathological features in CaOx-urolithiasis. In particular, the size decrease of the ureter, LU, and  
348 SM from Pro to Mid1 in healthy ureters and ADCF alternation in diseased ureters, including increased  
349 connective tissues with inflammation and decreased nerve bundles, were suggested to be a crucial  
350 to consider the pathogenesis of feline ureteral obstruction.

351

352 **Tables**

353 **Table 1. Body weights and ureter lengths in healthy cats.**

<b>Parameters</b>	<b>Value</b>
Body weight	4.32 ± 0.18 kg
Left ureter length	8.50 ± 0.35 cm
Right ureter length	9.88 ± 0.38 cm
Mean of left and right ureter lengths	9.19 ± 0.34 cm

Value = mean ± standard error. n = 4, male, mixed breed.

354

355

**Table 2. General and clinical information and ureter length in the cats showing urolithiasis.**

ID	Breed	Sex	Urolith type	Left Right ureter	or Age	Body weight	BUN	CRE	Dissected ureter length <sup>#</sup>
1	Somali	Cast	Calcium oxalate	Unknown	10.75	2.58	57.90	3.10	7.21
2	Mix	Cast	Calcium oxalate	Unknown	5.42	3.65	68.70	3.10	6.71
3	Mix	Cast	Calcium oxalate	Unknown	9.58	3.60	36.90	2.10	4.75
4	Ragdoll	Cast	Calcium oxalate	Left	4.92	3.60	136.80	6.30	6.87
5	Mix	Cast	Dried solidified blood stone, Calcium oxalate	Unknown	11.83	4.60	45.60	1.71	6.10
6	Scottish Fold	Cast	Calcium oxalate	Unknown	5.33	4.08	32.30	2.01	5.25
7	Munchkin	Cast	Calcium oxalate	Unknown	2.83	4.32	168.60	11.44	1.89
8	Mix*	Cast	Calcium oxalate	Left	1.83	4.30	76.90	5.15	5.58
9	Mix*	Cast	Calcium oxalate	Right	1.83	4.30	76.90	5.15	3.84
<b>Male</b>				<b>Mean</b>	<b>6.56</b>	<b>3.84</b>	<b>77.96</b>	<b>4.36</b>	<b>5.55</b>
				<b>SE</b>	<b>1.31</b>	<b>0.22</b>	<b>17.42</b>	<b>1.16</b>	<b>0.60</b>
10	Mix	Spay	Calcium oxalate	Right	2.75	2.78	35.00	1.45	6.60
11	Norwegian Forest	Spay	Magnesium phosphate	ammonium Unknown	10.58	5.80	41.30	2.60	11.00
12	Scottish Fold	Spay	Calcium oxalate	Right	11.75	2.00	140.00	13.80	4.67
13	Mix*	Spay	Calcium oxalate	Right	10.00	4.20	262.80	22.20	3.73
14	Mix*	Spay	Calcium oxalate	Left	10.00	4.20	262.80	22.20	3.73
15	Ragdoll	Spay	Calcium oxalate	Unknown	5.17	3.82	182.70	16.60	5.07
16	Scottish Fold*	Spay	Calcium oxalate	Left	7.75	3.50	140.00	11.50	8.27
17	Scottish Fold*	Spay	Calcium oxalate	Right	7.75	3.50	140.00	11.50	1.64
18	American Shorthair	Female	Calcium oxalate	Unknown	4.50	3.10	37.10	1.70	7.44
19	Mix	Spay	Calcium oxalate	Unknown	13.42	3.55	92.80	7.20	6.07
20	Russian Blue*	Spay	Calcium oxalate	Left	5.75	3.45	54.20	2.30	2.18
21	Russian Blue*	Spay	Calcium oxalate	Right	5.75	3.45	54.20	2.30	2.55
22	Mix	Spay	Calcium oxalate, Ammonium acid urate	Unknown	6.17	4.85	24.30	2.10	2.48
23	Mix	Spay	Calcium oxalate	Unknown	7.83	2.65	119.80	4.52	3.98
24	Mix	Spay	Calcium oxalate	Unknown	10.00	3.90	36.60	3.06	7.35
<b>Female</b>				<b>Mean</b>	<b>7.97</b>	<b>3.63</b>	<b>97.22</b>	<b>7.42</b>	<b>5.74</b>
				<b>SE</b>	<b>0.93</b>	<b>0.29</b>	<b>21.39</b>	<b>2.01</b>	<b>0.74</b>
<b>Male and Female</b>				<b>Mean</b>	<b>7.41</b>	<b>3.72</b>	<b>89.52</b>	<b>6.20</b>	<b>5.66</b>
				<b>SE</b>	<b>0.76</b>	<b>0.19</b>	<b>14.43</b>	<b>1.31</b>	<b>0.49</b>

\*: These ureters are analyzed in same cats. BUN: blood urea nitrogen. CRE: serum creatinine. SE: standard error. #: Ureters are dissected from obstructed lesion to junction with the urinary bladder.

357 **Figure legends**

358 **Figure 1. Histological features of the ureters in healthy cats**

359 **A:** Histology of the ureters. The ureters were examined in each segment, and defined as proximal  
360 (Pro), middle 1 (Mid1), middle 2 (Mid2), and distal (Dis). Masson's trichrome staining.

361 **B:** Indices of ureter perimeters examined in adventitia composed of collagen fibers (ADCF) or circular  
362 muscular layer (CL).

363 **C:** Area of each ureter component.

364 **D:** Area ratio of each ureter component expressed by percentage.

365 "Total" indicates the mean of all segments. Values = mean  $\pm$  standard error.  $n = 8$  ureters. Scheffe's  
366 method among Pro, Mid1, Mid2, and Dis following significance in the Kruskal-Wallis test. P, P\*:  
367 significance with Pro ( $P < 0.05$ ,  $P < 0.01$ ). LU: lumen. TE: transitional epithelium. SM: submucosa.

368

369 **Figure 2. Histological features of the ureter muscular layers in healthy cats**

370 **A:** Histology of ureters. The insets show magnification of the square areas of internal longitudinal  
371 muscular layer (ML) (Int LL)<sup>1</sup> and external longitudinal ML (Ext LL)<sup>2</sup>. Arrowheads: longitudinal  
372 muscular fibers. Arrows: longitudinal muscular fibers in adventitia composed of collagen fibers (Ad  
373 LL). Mid CL: middle circular ML. Dotted lines in insets: borders between Mid CL and Int LL or Ext LL.  
374 Masson's trichrome staining.

375 **B:** Semi-quantitative indices of ureter ML development. "Total" indicates the mean of all segments.

376 **C:** Semi-quantitative indices of ML development in each ureter component.

377 Values = mean  $\pm$  standard error.  $n = 8$  ureters. Scheffe's method following significance in the Kruskal-  
378 Wallis test. IL, ML, EL, AL: significance with Int LL, Mid CL, Ext LL, and Ad LL ( $P < 0.05$ ) (B). P: proximal.  
379 M1: middle 1. M2: middle 2. D: distal P, M1: significance with P, M1 ( $P < 0.05$ ) (C). Asterisks in addition  
380 to the letters indicate high significance ( $P < 0.01$ ) (B and C).

381

382 **Figure 3. Histopathological features of cat ureters showing urolithiasis**

383 **A:** Resected ureters in cats with urolithiasis. Ureters were examined by dividing them into four equal  
384 parts termed "disease parts (dParts) 1-4". Gross anatomical features.

385 **B and C:** Histopathology of ureters in cats with urolithiasis. Dilated (B) and fibrotic (C) cases. Masson's  
386 trichrome staining.

387 **D:** Indices of ureter perimeters examined in adventitia composed of collagen fibers (ADCF) or circular  
388 muscular layer (CL).

389 **E:** Area of each ureter component.

390 **F:** Area ratio of each ureter component expressed by percentage.

391 Values = mean  $\pm$  standard error.  $n = 24$  ureters. Dunnett's test with dPart1 following significance in  
392 the Kruskal-Wallis test. P4: significance with dPart4 ( $P < 0.05$ ) (D and E). LU: lumen. TE: transitional  
393 epithelium. SM: submucosa.

394

#### 395 **Figure 4. Perimeters of cat ureters showing urolithiasis**

396 **A:** Illustration for diseased ureter analysis. Diseased ureters were analyzed by defining each obtained  
397 part to proximal (Pro), middle 1 (Mid1), middle 2 (Mid2), and distal (Dis), according to the distance  
398 from the ureter-urinary bladder junction using the segment definition of healthy cat samples.  
399 Diseased ureters containing an obstructed region (dUOR) and diseased ureters distal from the  
400 obstructed region (dUDR) were examined separately. "Type" indicates an example for each type of  
401 examined samples.

402 **B:** Indices of ureter perimeters examined in the adventitia composed of collagen fibers (ADCF) or  
403 circular muscular layer (CL). "Total" indicates the mean of all segments. Values = mean  $\pm$  standard  
404 error.  $n = 8$  (healthy ureters) and 24 (diseased ureters). Mann-Whitney  $U$ -test. \*\*: Significance with  
405 healthy ureter ( $P < 0.01$ ).

406 **C:** Indices of ureter perimeters examined in each segment. Values = mean  $\pm$  standard error.  $n$  (ureter  
407 number for healthy, dUOR, dUDR) = 8, 5, 0 (Pro); 8, 9, 10 (Mid1); 8, 6, 24 (Mid2); 8, 3, 35 (Dis). Mann-  
408 Whitney  $U$ -test (Pro). Scheffé's method following significance in the Kruskal-Wallis test (Mid1, Mid2,  
409 Dis) among healthy, dUOR, and dUDR. \*, \*\*: Significance with healthy ureter ( $P < 0.05$ ,  $P < 0.01$ ).

410

#### 411 **Figure 5. Area of each ureter component in the cat showing urolithiasis.**

412 **A:** Area of each ureter component.

413 **B:** Area ratio of each ureter component expressed by percentage.

414 “Total” indicates the mean of all segments. Values = mean  $\pm$  SE.  $n = 8$  (healthy ureters) and  $n = 24$   
415 (diseased ureters). Mann–Whitney  $U$ -test. \*, \*\*: Significance with healthy ureter ( $P < 0.05, 0.01$ ).

416 **C:** Area of each ureter component in each segment.

417 **D:** Area ratio of each ureter component expressed by percentage in each segment.

418 Each obtained part was defined as proximal (Pro), middle 1 (Mid1), middle 2 (Mid2), and distal (Dis),  
419 according to the distance from the ureter-urinary bladder junction using to the segment definition  
420 of healthy cat samples. Diseased ureters containing an obstructed region (dUOR) and diseased  
421 ureters distal from the obstructed region (dUDR) were examined separately.

422 Values = mean  $\pm$  standard error.  $n$  (ureter number for healthy (HC), dUOR, dUDR) = 8, 5, 0 (Pro); 8, 9,  
423 10 (Mid1); 8, 6, 24 (Mid2); 8, 3, 35 (Dis). Mann–Whitney  $U$ -test (Pro). Scheffé's method following  
424 significance in the Kruskal–Wallis test (Mid1, Mid2, Dis) among HC, dUOR, and dUDR. \*, \*\*:   
425 Significance with HC ( $P < 0.05, P < 0.01$ ). LU: lumen. TE: transitional epithelium. SM: submucosa. CL:  
426 circular muscular layer. ADCF: adventitia composed of collagen fibers.

427

#### 428 **Figure 6. Muscular layer of the ureter in cats showing urolithiasis**

429 **A:** Semi-quantitative indices of ureter muscular layer (ML) development. “Total” indicates the mean  
430 of all segments. Values = mean  $\pm$  standard error.  $n = 8$  (healthy ureters) and 24 (diseased ureters).  
431 Mann–Whitney  $U$ -test. \*, \*\*: Significance with healthy ureter ( $P < 0.05, 0.01$ ).

432 **B:** Semi-quantitative indices of ureter ML development in each segment.

433 Each obtained part was defined as proximal (Pro), middle 1 (Mid1), middle 2 (Mid2), and distal (Dis),  
434 according to the distance from the ureter-urinary bladder junction using the segment definition of  
435 healthy cat samples. Diseased ureters containing an obstructed region (dUOR) and diseased ureters  
436 distal from the obstructed region (dUDR) were examined separately.

437 Values = mean  $\pm$  standard error.  $n$  (ureter number for healthy, dUOR, dUDR) = 8, 5, 0 (Pro); 8, 9, 10  
438 (Mid1); 8, 6, 24 (Mid2); 8, 3, 35 (Dis). Mann–Whitney  $U$ -test (Pro). Scheffé's method following  
439 significance in the Kruskal–Wallis test (Mid1, Mid2, Dis). \*, \*\*: Significance with healthy ureter ( $P <$   
440  $0.05, P < 0.01$ ). ##: Significance with dUDR ( $P < 0.01$ ).

441

#### 442 **Figure 7. Inflammation of the ureter in the cat showing urolithiasis**

443 **A:** Localization of CD20<sup>+</sup> B cells and IBA1<sup>+</sup> macrophages in the diseased ureters.  
444 Immunohistochemistry. LU: lumen. TE: transitional epithelium. SM: submucosa. CL: circular  
445 muscular layer. ADCF: adventitia composed of collagen fibers.

446 **B:** Number of CD20<sup>+</sup> B cells and IBA1<sup>+</sup> macrophages in each ureter. Ureters were examined by dividing  
447 them into four equal parts termed “disease parts (dParts) 1–4”. Values = mean ± standard error. *n* =  
448 24 ureters.

449 **C:** Number of CD20<sup>+</sup> B cells and IBA1<sup>+</sup> macrophages in each ureter. Ureters were examined by dividing  
450 them into four equal parts termed “dParts 1–4”. “Total” indicates the mean of all segments. Values  
451 = mean ± standard error. *n* = 8 (healthy ureters) and 24 (diseased ureters). Mann–Whitney *U*-test. \*,  
452 \*\*Significance with healthy ureter (*P* < 0.01).

453 **D:** Number of CD20<sup>+</sup> B cells in each ureter segment.

454 **E:** Number of IBA1<sup>+</sup> macrophages in each ureter segment.

455 Each obtained part was defined as proximal (Pro), middle 1 (Mid1), middle 2 (Mid2), and distal (Dis),  
456 according to the distance from the ureter-urinary bladder junction using the segment definition of  
457 healthy cat samples. Diseased ureters containing an obstructed region (dUOR) and diseased ureters  
458 distal from the obstructed region (dUDR) were examined separately.

459 Values = mean ± standard error. *n* (ureter number for healthy (HC), dUOR, dUDR) = 8, 5, 0 (Pro); 8, 9,  
460 10 (Mid1); 8, 6, 24 (Mid2); 8, 3, 35 (Dis). Mann–Whitney *U*-test (Pro). Scheffé's method following  
461 significance in the Kruskal–Wallis test (Mid1, Mid2, Dis) among HC, dUOR, and dUDR. \*, \*\*:   
462 Significance with HC (*P* < 0.05, *P* < 0.01).

463

464 **Figure 8. Histopathological characteristics found in the ureter of cats showing urolithiasis.**

465 **A:** Nerve bundles found in the ureter adventitia in healthy and diseased cats. Arrows: nerve bundles.  
466 Masson's trichrome staining.

467 **B:** Number of nerve bundles in each ureter. The ureters were examined by dividing them into four  
468 equal parts termed “disease parts (dParts) 1–4”, left panel. The right panel indicates the mean of all  
469 the parts. Values = mean ± standard error (*n* = 24 ureters). Mann–Whitney *U*-test. \*\*: Significance  
470 with healthy ureter (*P* < 0.01).

471 **C:** Number of nerve bundles in each ureter segment.

472 Each obtained part was defined as proximal (Pro), middle 1 (Mid1), middle 2 (Mid2), and distal (Dis),  
473 according to the distance from the ureter-urinary bladder junction using the segment definition of  
474 healthy cat samples. Diseased ureters containing an obstructed region (dUOR) and diseased ureters  
475 distal from the obstructed region (dUDR) were examined separately.

476 Values = mean  $\pm$  standard error. *n* (ureter number for healthy (HC), dUOR, dUDR) = 8, 5, 0 (Pro); 8, 9,  
477 10 (Mid1); 8, 6, 24 (Mid2); 8, 3, 35 (Dis). \*\*Significance with HC ( $P < 0.01$ ). Scheffé's method following  
478 significance in the Kruskal–Wallis test (Mid1, Mid2, Dis).

479 **D:** Characteristic features found in ureters of cats with urolithiasis. Transitional epithelium (TE)  
480 decudation (upper panels), TE invasion (lower left panel), and mucosal fold formation (lower right  
481 panel) were observed. Masson's trichrome staining.

482

483 **Author Contributions**

484 OI, KO, and YK conceptualized the study design. OI designed the experiments. OI, KO, HM, NY, TaN,  
485 YO, and NS performed the sampling and experiments. OI and TeN analyzed the data. All authors  
486 reviewed and discussed the results and contributed to the preparation of the manuscript. YK  
487 supervised the project.

488

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490 None.

491

492 **Data Availability**

493 The data that support the findings of this study are available from the corresponding author upon  
494 reasonable request.

495

496 **Disclosures**

497 The authors declare no competing interests.

498

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501

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# Table 1

<b>Parameters</b>	<b>Value</b>
Body weight	4.32 ± 0.18 kg
Left ureter length	8.50 ± 0.35 cm
Right ureter length	9.88 ± 0.38 cm
Mean of left and right ureter length	9.19 ± 0.34 cm

Value = mean ± standard error. n = 4, male, mixed breed.

Figure 1

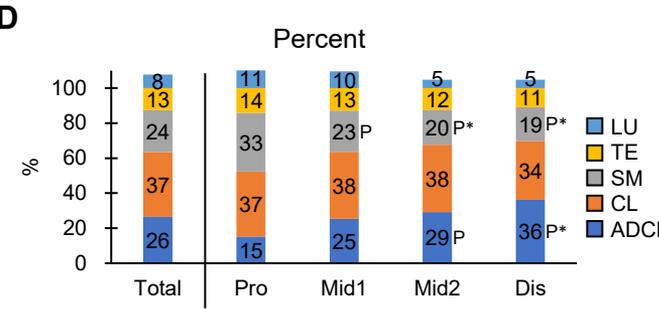
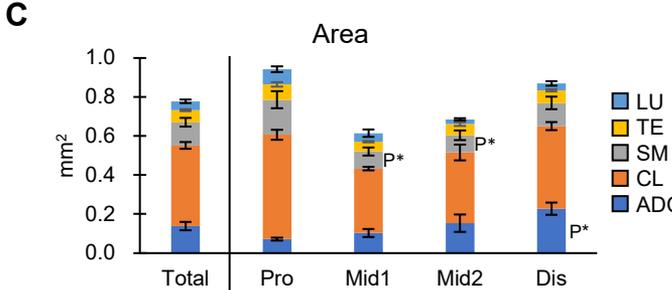
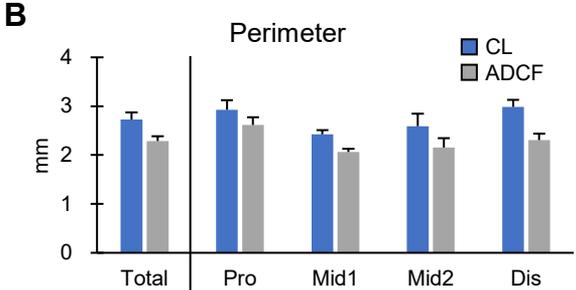
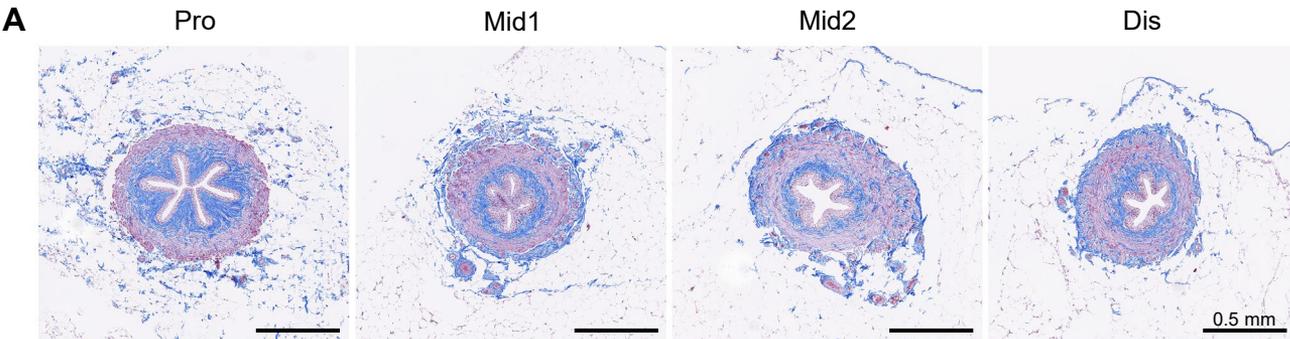


Figure 2

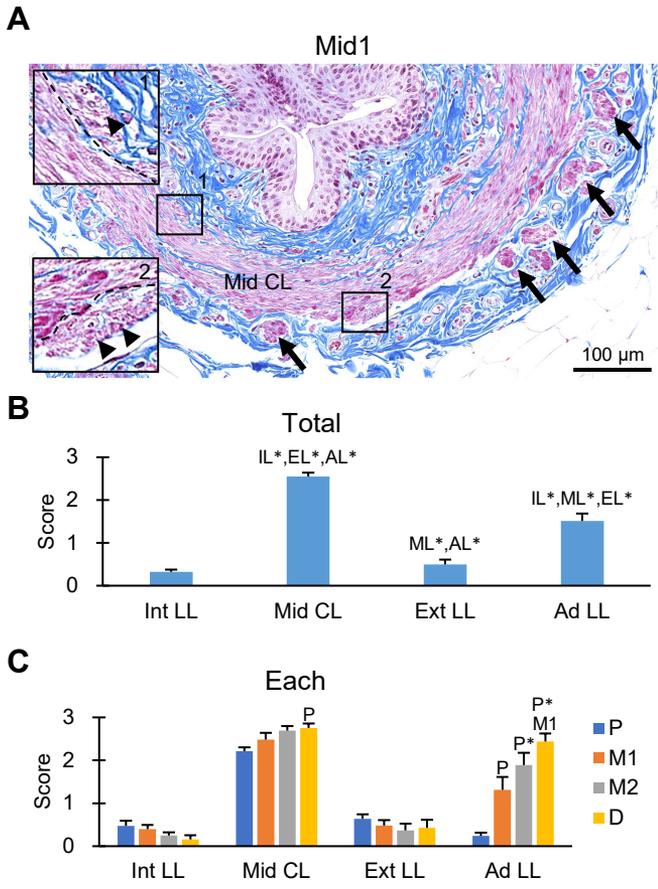


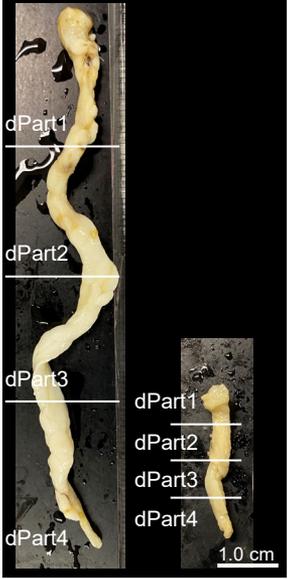
Table 2

ID	Breed	Sex	Urolith type	Left or Right ureter	Age	Body weight	BUN	Cre	Dissected ureter length <sup>#</sup>	
1	Somali	Cast	Calcium oxalate	Unknown	10.75	2.58	57.90	3.10	7.21	
2	Mix	Cast	Calcium oxalate	Unknown	5.42	3.65	68.70	3.10	6.71	
3	Mix	Cast	Calcium oxalate	Unknown	9.58	3.60	36.90	2.10	4.75	
4	Ragdoll	Cast	Calcium oxalate	Left	4.92	3.60	136.80	6.30	6.87	
5	Mix	Cast	Dried solidified blood stone, Calcium oxalate	Unknown	11.83	4.60	45.60	1.71	6.10	
6	Scottish Fold	Cast	Calcium oxalate	Unknown	5.33	4.08	32.30	2.01	5.25	
7	Munchkin	Cast	Calcium oxalate	Unknown	2.83	4.32	168.60	11.44	1.89	
8	Mix*	Cast	Calcium oxalate	Left	1.83	4.30	76.90	5.15	5.58	
9	Mix*	Cast	Calcium oxalate	Right	1.83	4.30	76.90	5.15	3.84	
				<b>Male</b>	<b>Mean</b>	<b>6.56</b>	<b>3.84</b>	<b>77.96</b>	<b>4.36</b>	<b>5.55</b>
					<b>SE</b>	<b>1.31</b>	<b>0.22</b>	<b>17.42</b>	<b>1.16</b>	<b>0.60</b>
10	Mix	Spay	Calcium oxalate	Right	2.75	2.78	35.00	1.45	6.60	
11	Norwegian Forest	Spay	Magnesium ammonium phosphate	Unknown	10.58	5.80	41.30	2.60	11.00	
12	Scottish Fold	Spay	Calcium oxalate	Right	11.75	2.00	140.00	13.80	4.67	
13	Mix*	Spay	Calcium oxalate	Right	10.00	4.20	262.80	22.20	3.73	
14	Mix*	Spay	Calcium oxalate	Left	10.00	4.20	262.80	22.20	3.73	
15	Ragdoll	Spay	Calcium oxalate	Unknown	5.17	3.82	182.70	16.60	5.07	
16	Scottish Fold*	Spay	Calcium oxalate	Left	7.75	3.50	140.00	11.50	8.27	
17	Scottish Fold*	Spay	Calcium oxalate	Right	7.75	3.50	140.00	11.50	1.64	
18	American Shorthair	Female	Calcium oxalate	Unknown	4.50	3.10	37.10	1.70	7.44	
19	Mix	Spay	Calcium oxalate	Unknown	13.42	3.55	92.80	7.20	6.07	
20	Russian Blue*	Spay	Calcium oxalate	Left	5.75	3.45	54.20	2.30	2.18	
21	Russian Blue*	Spay	Calcium oxalate	Right	5.75	3.45	54.20	2.30	2.55	
22	Mix	Spay	Calcium oxalate, Ammonium acid urate	Unknown	6.17	4.85	24.30	2.10	2.48	
23	Mix	Spay	Calcium oxalate	Unknown	7.83	2.65	119.80	4.52	3.98	
24	Mix	Spay	Calcium oxalate	Unknown	10.00	3.90	36.60	3.06	7.35	
				<b>Female</b>	<b>Mean</b>	<b>7.97</b>	<b>3.63</b>	<b>97.22</b>	<b>7.42</b>	<b>5.74</b>
					<b>SE</b>	<b>0.93</b>	<b>0.29</b>	<b>21.39</b>	<b>2.01</b>	<b>0.74</b>
				<b>Male and Female</b>	<b>Mean</b>	<b>7.41</b>	<b>3.72</b>	<b>89.52</b>	<b>6.20</b>	<b>5.66</b>
					<b>SE</b>	<b>0.76</b>	<b>0.19</b>	<b>14.43</b>	<b>1.31</b>	<b>0.49</b>

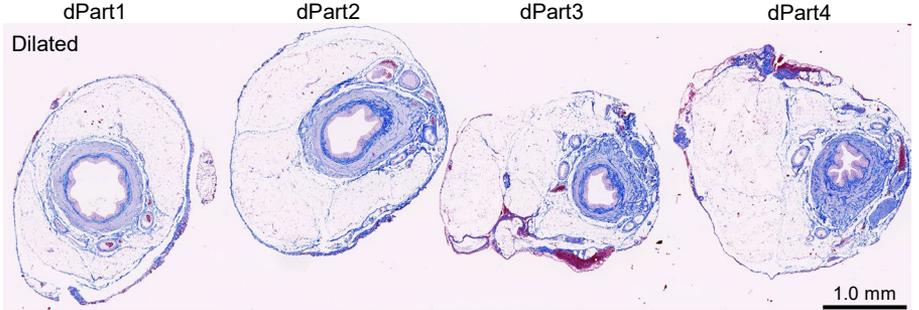
\*: These ureters are analyzed in same cats. BUN: blood urea nitrogen. Cre: serum creatinine. SE: standard error. #: Ureters are dissected from obstructed lesion to junction with the urinary bladder.

Figure 3

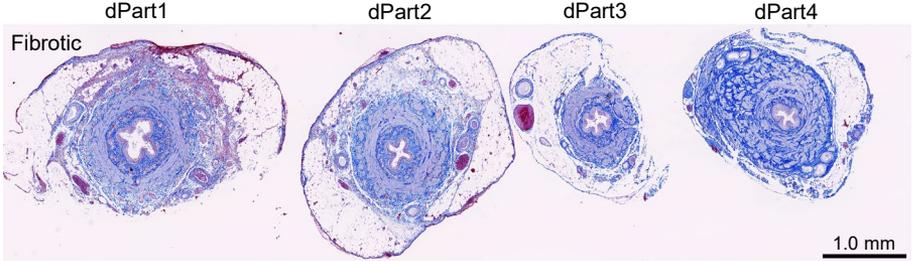
**A**



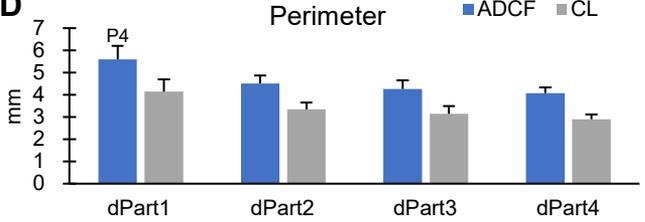
**B**



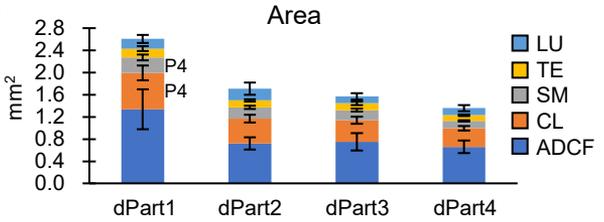
**C**



**D**



**E**



**F**

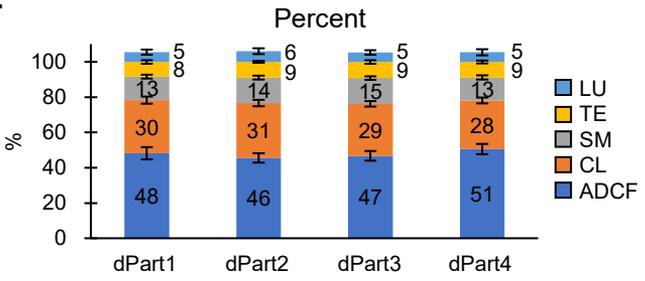


Figure 4

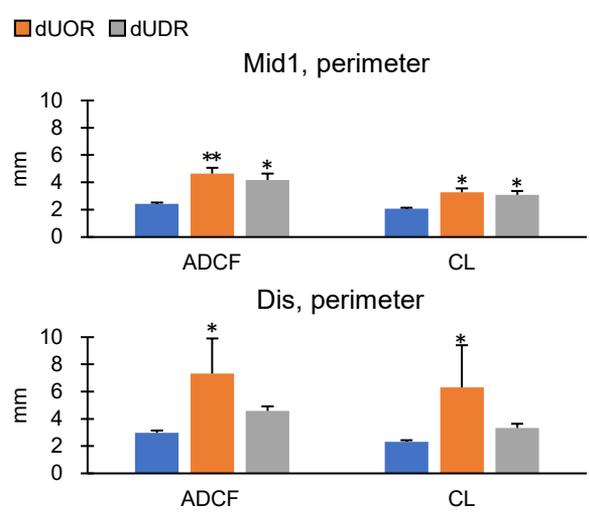
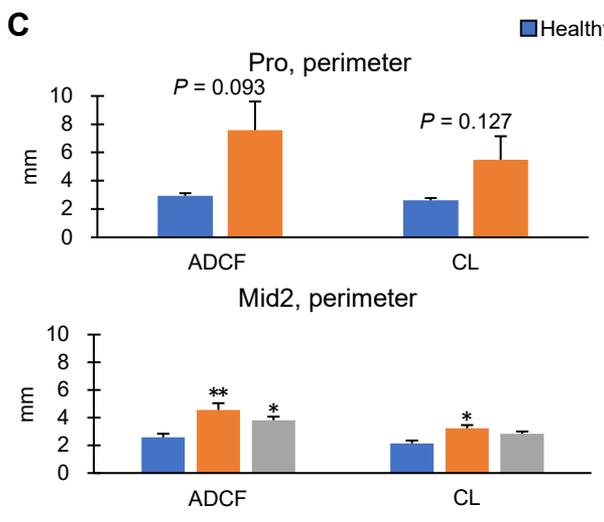
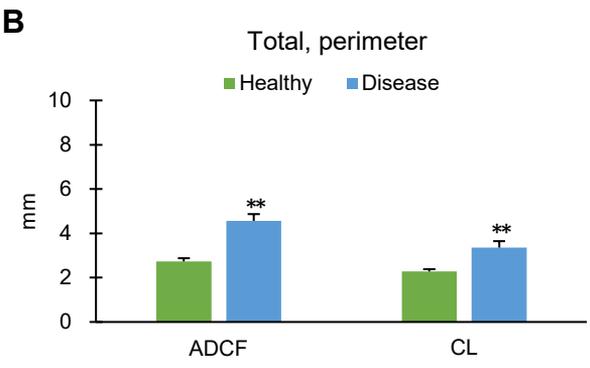
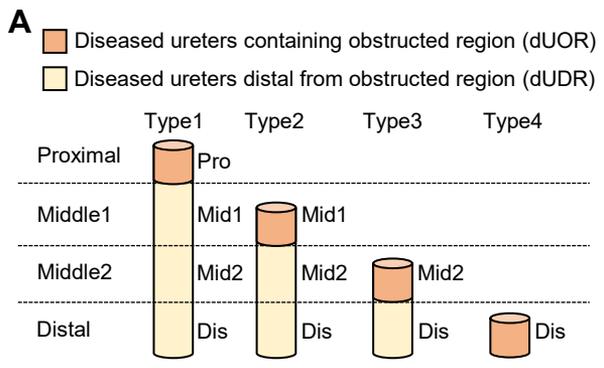


Figure 5

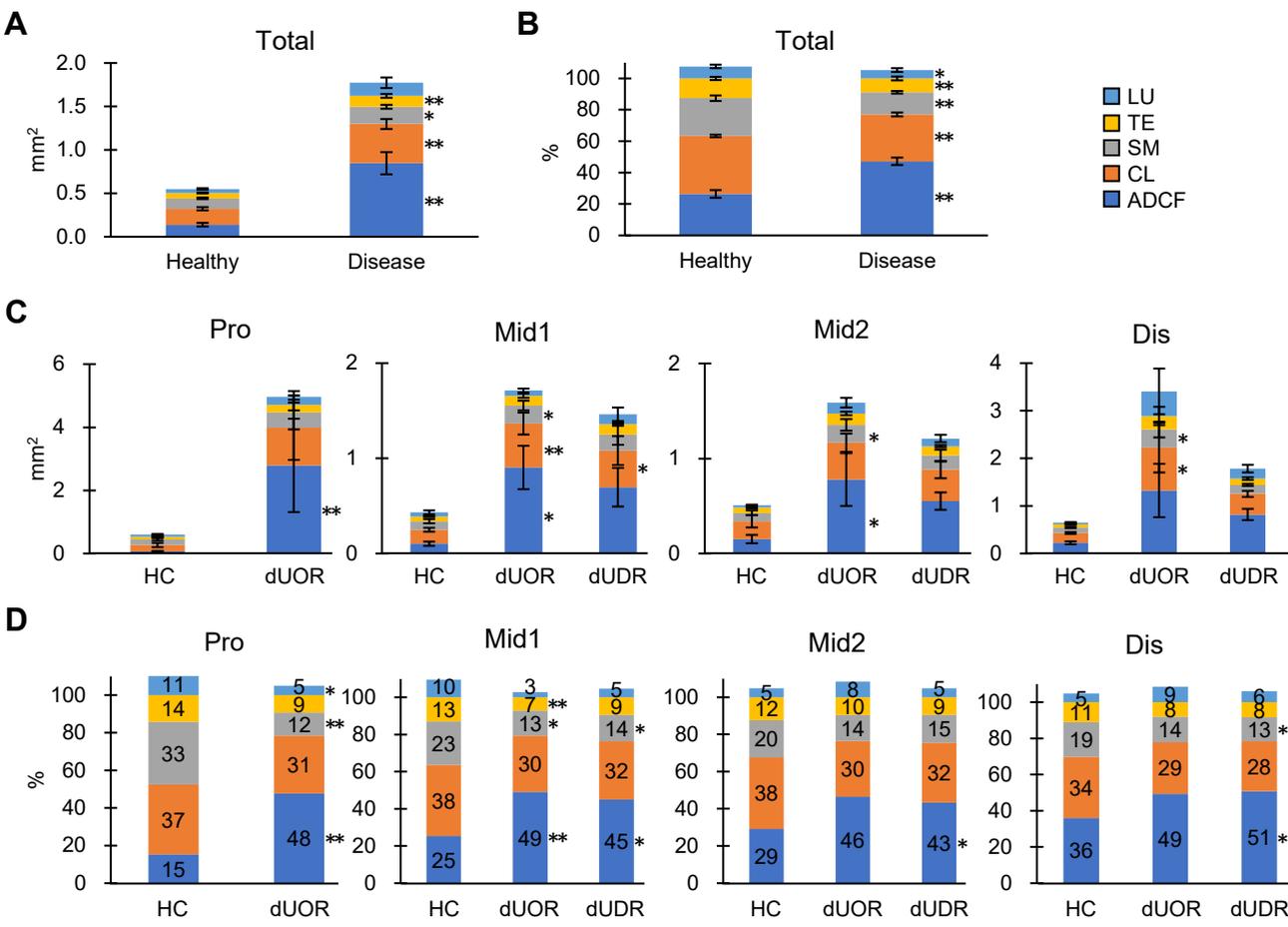
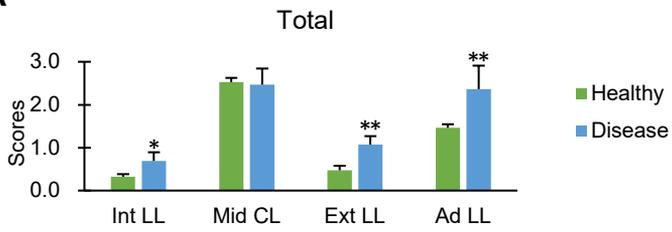


Figure 6

**A**



**B**

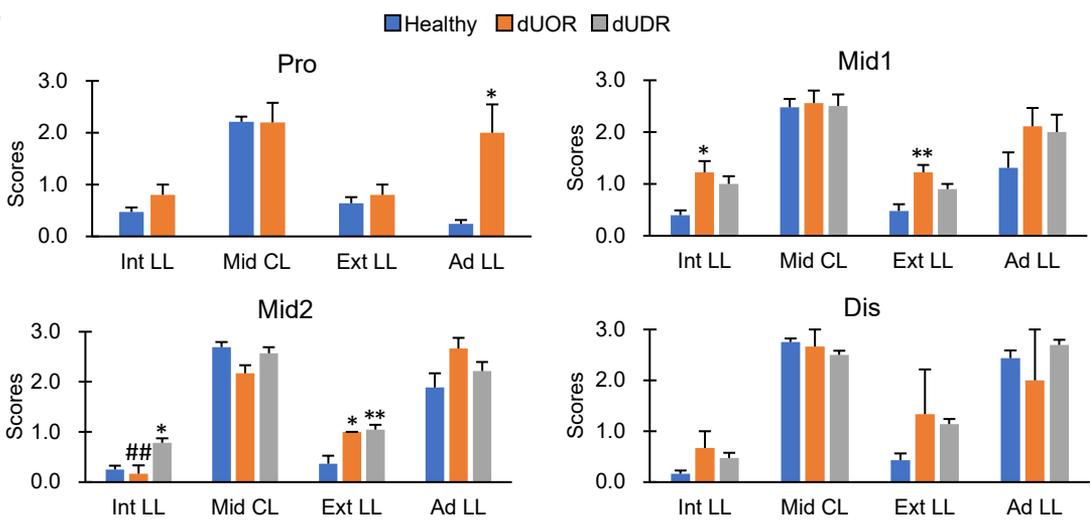


Figure 7

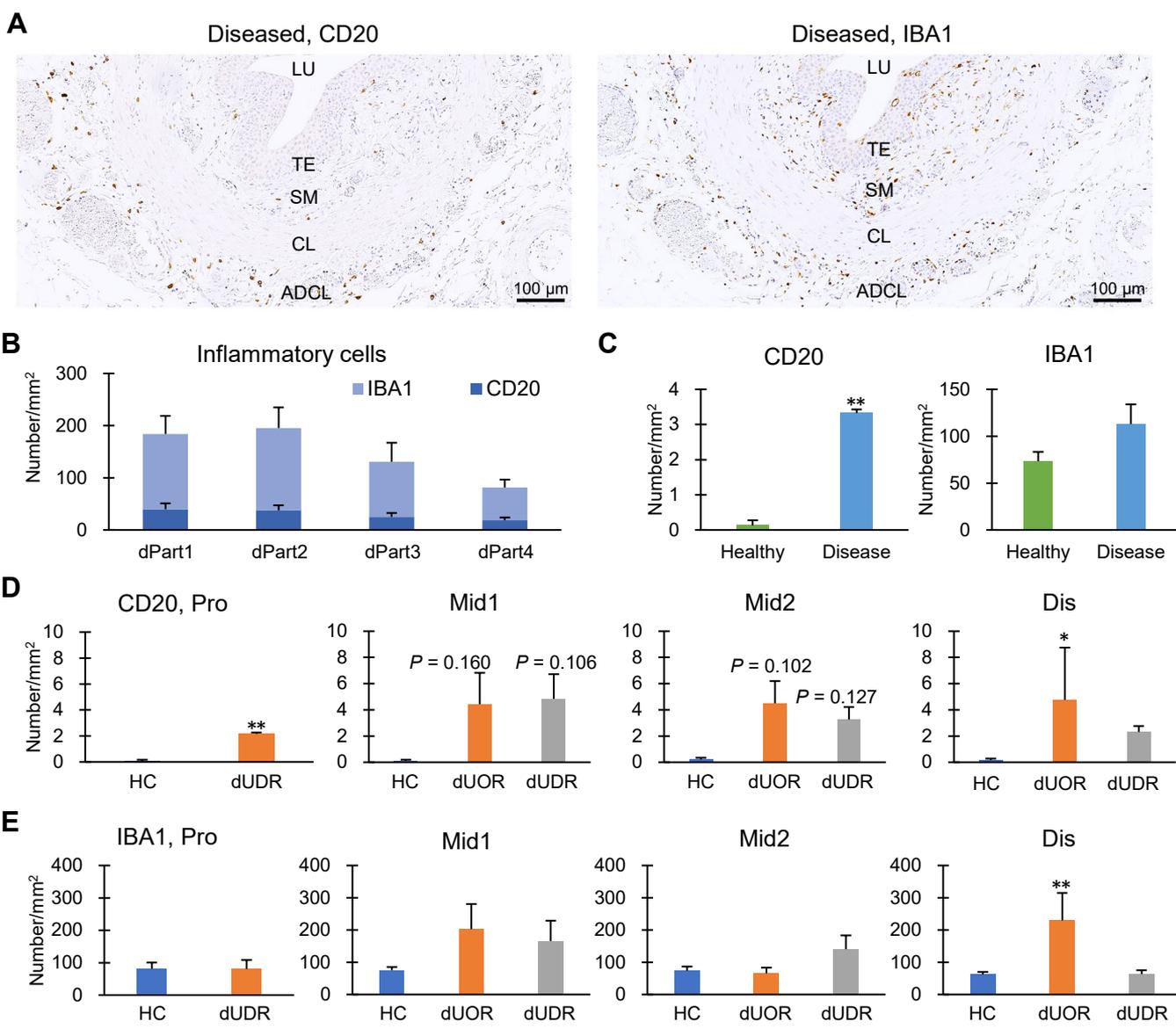


Figure 8

